APPENDIX 3.3.1

# *IN VIVO* DERIVED EMBRYOS

Article 3.3.1.1.

**Aims of control**

The purpose of official sanitary control of *in vivo* derived embryos intended for movement internationally is to ensure that specific pathogenic organisms, which could be associated with embryos, are controlled and transmission of infection to recipient animals and progeny is avoided.

Article 3.3.1.2.

**Conditions applicable to the embryo collection team**

**The embryo collection team is a group of competent technicians, including at least one veterinarian, to perform the collection, processing and storage of embryos. The following conditions should apply:**

1. **The team should be supervised by a team veterinarian.**

2. **The team veterinarian is responsible for all team operations which include verification of donor health status, sanitary handling and surgery of donors and *disinfection* and hygienic procedures.**

3. **The team veterinarian should be specifically approved for this purpose by an *Official Veterinarian*.**

4. **Team personnel should be adequately trained in the techniques and principles of disease control. High standards of hygiene should be practiced to preclude the introduction of infection.**

5. The collection team must have adequate facilities and equipment for:

   a) collecting embryos;

   b) processing and treatment of embryos at a permanent site or mobile laboratory;

   c) storing embryos.

   These facilities need not necessarily be at the same location.

6. The collection team must keep a record of its activities, which must be maintained for inspection by the approving authority for a period of at least two years after the embryos have been exported.

7. The collection team should be subjected to [regular] inspection at least once a year by an *Official Veterinarian* to ensure compliance with sanitary collection, processing and storage of embryos.

8. The collection team must not operate in an *infected zone* unless the disease in question has been listed by the International Embryo Transfer Society (IETS) as one on which sufficient research has been done to demonstrate that the risk of its transmission by embryo transfer is negligible.*

Article 3.3.1.3.

## Conditions applicable to the processing laboratories

The processing laboratory used by the embryo collection team may be mobile or permanent. It is a facility in which embryos are recovered from collection media, examined and subjected to any required treatments such as washing before freezing, storage and quarantine, pending results of diagnostic procedures.

A permanent laboratory may be part of a specifically designed collection and processing unit, or a suitably adapted part of an existing building. It may be on the premises where the donor animals are kept. In either case, the laboratory should be physically separated from animals. Both mobile and permanent laboratories should have a clear separation between dirty areas (animal handling) and the clean processing area.

Additionally:

1. The laboratory should be under the direct supervision of the team veterinarian and regularly inspected by an *Official Veterinarian*.

2. While embryos for export are being handled prior to their storage in ampules, vials or straws, no embryos of a lesser health status should be processed.

3. The laboratory should be protected against rodents and insects.

4. The processing laboratory should be constructed with materials which permit its effective cleansing and *disinfection*. This should be done following each occasion on which embryos are processed.

5. The laboratory must not be situated in an *infected zone* unless the disease in question has been listed by the IETS as one on which sufficient research has been done to demonstrate that the risk of its transmission by embryo transfer is negligible*.

Article 3.3.1.4.

## Conditions applicable to the introduction of donor animals

1. Donor animals

   a) The *Veterinary Administration* should have knowledge of, and authority over, the herd/flock of origin of the donor animals.

   b) At the time of collection, donor animals should be clinically inspected by a veterinarian responsible to the team veterinarian and certified to be free of clinical signs of contagious and infectious diseases transmissible by washed embryos to the species concerned.

   c) The herd of origin must not be situated in an *infected zone* for the 30 days before and after embryo collection unless the disease in question has been listed by the IETS as one on which sufficient research has been done to demonstrate that the risk of its transmission by embryo transfer is negligible*.

d) The donor animals should not have been imported from another country during the previous 60 days and should have been in the herd of origin for at least 30 days prior to collection.

2. Semen donors

   a) Semen used to inseminate donor animals artificially should have been produced and processed in accordance with the provisions of Appendix 3.2.1., Appendix 3.2.2. or Appendix 3.2.3. as relevant.

   b) When the donor of the semen used to inseminate donor females for embryo production is no longer living, and when the health status of the semen donor concerning a particular infectious disease or diseases of concern was not known at the time of semen collection, additional tests may be required of the inseminated donor female after embryo collection to verify that these infectious diseases were not transmitted. An alternative may be to subject an aliquot of semen from the same collection date to testing.

   c) Where natural service or fresh semen is used, donor sires should meet the same health requirements as donor females.

Article 3.3.1.5.

**Risk management**

With regard to disease transmission, transfer of *in vivo* derived embryos is a very low risk method for moving animal genetic material. Irrespective of animal species, there are three phases in the embryo transfer process that determine the final level of risk**:

1. The first phase comprises the potential for embryo contamination and depends on:

   a) the disease situation in the *exporting country* and/or zone;

   b) the health status of the herds/flocks and the donors from which the embryos are collected;

   c) the pathogenic characteristics of the specified disease agents.

2. The second phase covers risk mitigation by use of internationally accepted procedures for processing of embryos which are set out in the IETS Manual***. These include the following:

   a) The embryos must be washed at least ten times with at least 100-fold dilutions between each wash, and a fresh pipette for transferring the embryos through each wash.

   b) Only embryos from the same donor should be washed together.

   c) Sometimes, for example when inactivation or removal of certain viruses (e.g. bovine herpesvirus-1, and Aujeszky's disease virus) is required, the standard washing procedure should be modified to include additional washes with the enzyme trypsin, as described in the IETS Manual***.

   d) The zona pellucida of each embryo, after washing, must be examined over its entire surface area at not less than 50X magnification to ensure that it is intact and free of adherent material.

*[NOTE: All shipments of embryos must be accompanied by a statement signed by the team veterinarian certifying that these embryo processing procedures have been completed.]*

3. The third phase, which is applicable to animal pathogens not included in category 1 of the IETS classification, encompasses the risk reductions resulting from:

   a) post-collection surveillance of the donors and donor herds based on the recognized *incubation periods* of the diseases of concern to determine retrospectively the health status of donors whilst the embryos are stored (in species where effective cryopreservation is possible) in the *exporting country*;

   b) testing of embryo-collection (flushing) fluids and non-viable embryos, or other samples such as blood, for presence of specified disease agents.

Article 3.3.1.6.

**Conditions applicable to the collection and storage of embryos**

1. Media

   Any biological product of animal origin used in the media and solutions for collection, processing, washing or storage of embryos should be free of pathogenic micro-organisms. Media and solutions used in the collection, freezing and storage of embryos should be sterilized by approved methods according to the IETS Manual*** and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to collection, processing, washing and storage media as recommended in the IETS Manual***.

2. Equipment

   a) All equipment used to collect, handle, wash, freeze and store embryos should be sterilized prior to use as recommended in the IETS Manual***.

   b) Used equipment should be transferred between countries for re-use by the embryo collection team only if cleaning and *disinfection* procedures appropriate to the disease risk concerned are followed.

Article 3.3.1.7.

**Optional tests and treatments**

1. The examination of embryos and collection or washing fluids can be requested by an *importing country*. Tests may be carried out on these samples to confirm the absence of pathogenic organisms, or to assess whether the degree of quality control of the collection team is at an acceptable level:

   a) Embryos/oocytes

      Where the viable, zona intact embryos are intended for export, all non-fertilized oocytes and degenerated or zona compromised embryos collected from a donor should be washed according to the IETS Manual*** and pooled for possible testing. Only embryos/oocytes from one donor should be processed simultaneously.

b) Collection fluids

The collection fluid should be placed in a sterile, closed container and, if there is a large amount, it should be allowed to stand undisturbed for one hour. The supernatant fluid should then be removed and the bottom 10-20 ml, along with accumulated debris, decanted into a sterile bottle. If a filter is used in the collection of embryos/oocytes, then any debris that is retained on the filter must be rinsed into the retained fluid.

c) Washing fluids

The last four washes of the embryos/oocytes (washes 7, 8, 9 and 10) should be pooled (IETS Manual***).

d) Samples

The samples referred to above should be stored at 4°C and tested within 24 hours. If this is not possible, then samples should be stored frozen at -70° C or lower.

2. When treatment of the viable embryos is modified to include additional washings with the enzyme trypsin (see paragraph 2c) in Article 3.3.1.5.), the procedure should be carried out according to the IETS Manual***. It should be noted that such enzymatic treatment is not necessarily always beneficial and it should not be regarded as a general disinfectant. It may also have adverse effects on embryo viability, for instance in the case of equine embryos where the embryonic capsule could be damaged by the enzyme.

Article 3.3.1.8.

**Conditions applicable to the storage, quarantine and transport of embryos**

1. Embryos should be frozen in fresh liquid nitrogen and then stored in fresh liquid nitrogen in cleaned and disinfected tanks or containers.

2. The embryos should be stored in sealed sterile ampoules, vials or straws under strict hygienic conditions at a storage place approved by the *Veterinary Administration* of the *exporting country* where there is no risk of contamination of the embryos.

3. Only embryos from the same donor should be stored together in the same ampoule, vial or straw.

4. Ampoules, vials or straws must be sealed at the time of freezing (or prior to export where cryopreservation is not possible), and they should be clearly identified by labels according to the standardised system recommended in the IETS Manual***.

5. Liquid nitrogen containers should be sealed prior to shipment from the *exporting country*.

6. Embryos must not be exported until the appropriate health certification documents are completed.

Article 3.3.1.9.

Specific conditions applicable to bovine embryos

**[1.]** The herd of origin should be free of clinical signs of **[foot and mouth disease,]** rinderpest and contagious bovine pleuropneumonia.

**[2. The herd of origin should preferably be within a national disease control programme and at least free of**

**brucellosis and tuberculosis.]**

Article 3.3.1.10.

Specific conditions applicable to porcine embryos

**[1.]** The herd of origin should be free of clinical signs of foot and mouth disease, African swine fever, swine vesicular disease, classical swine fever, Aujeszky's disease and pathogenic enterovirus encephalomyelitis.

**[2. The herd of origin should preferably be within a national disease control programme and at least free of brucellosis.]**

*[NOTE: The development of effective cryopreservation methods for zona pellucida-intact porcine embryos is still at a very early stage.]*

Article 3.3.1.11.

Specific conditions applicable to ovine/caprine embryos

**[1.]** The herd of origin should be free of clinical signs of foot and mouth disease, peste des petits ruminants, sheep/goat pox and bluetongue.

**[2. The herd of origin should preferably be within a national disease control programme and at least free of brucellosis.]**

Article 3.3.1.12.

Specific conditions applicable to equine embryos

The recommendations apply principally to embryos from animals continuously resident in national equine populations and therefore may be found to be unsuitable for those from equines routinely involved in events or competitions at the international level. For instance, in appropriate circumstances horses travelling with an *international veterinary certificate* (e.g. competition horses) may be exempt from this condition where mutually agreed upon on a bilateral basis between the respective *Veterinary Administrations*.

The herd of origin should be free of clinical signs of OIE listed contagious diseases, such as African horse sickness.

The herd of origin should preferably be within a national disease control programme and at least free from equine infectious anaemia.

Article 3.3.1.13.

Specific conditions applicable to camelid embryos

South American camelid embryos recovered from the uterine cavity by the conventional non-surgical flushing technique at 6.5 to 7 days post-ovulation are almost invariably at the hatched blastocyst stage, and thus the zona pellucida has already been shed. Since the embryos do not enter the uterus and cannot be recovered before 6.5 to 7 days. It must also be noted that pathogen interaction studies with South American camelid embryos have not yet been carried out.

The herd of origin should be free of clinical signs of foot and mouth disease, rinderpest, vesicular stomatitis, bluetongue and peste des petits ruminants.

The herd of origin should preferably be within a national disease control programme and at least free of brucellosis and tuberculosis.

The herd of origin must not be situated in an infected zone for the 60 days before and after embryo collection. This extention of the normal 30-day period is based on the lack of clear scientific data relevant to the species.

The donor animals should not have been imported from another country during the previous 6 months and should have been in the herd of origin for at least 60 days prior to collection. These extentions from the normal 60-day and 30-day periods, respectively, is based on the lack of clear scientific data relevant to the species.

*[NOTE: The development of cryopreservation methods for camelid embryos is still at a very early stage.]*

Article 3.3.1.14.

Specific conditions applicable to cervid embryos

The recommendations apply principally to embryos derived from animals continuously resident in national domestic or ranched cervid populations and therefore may be found to be unsuitable for those from cervids in feral or other circumstances related to biodiversity or germplasm conservation efforts.

The herd of origin should preferably be within a national disease control programme and at least free of brucellosis and tuberculosis.

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* Based on available research and field information, the Research Subcommittee of the International Embryo Transfer Society has categorized a number of pathogens by species based on their relative risk of dissemination by properly processed and handled *in vivo* derived embryos as published in the *Rev. Sci. Tech. Off. Int. Epiz.*, 1998, **17** (3), 839-843. At the present time, there are no diseases of equines, South American camelids or cervids included in the four categories established by the Research Subcommittee of the International Embryo Transfer Society.

** SUTMOLLER P. & WRATHALL A.E. (1997) - A quantitative assessment of the risk of transmission of foot-and-mouth disease, bluetongue and vesicular stomatitis by embryo transfer in cattle. *Prev. Vet. Med.*, **32**, 111-132.

SUTMOLLER P. & WRATHALL A.E. (1997) - The risk of disease transmission by embryo transfer in cattle. *Rev. sci. tech. Off. Int. Epiz.*, **16** (1), 226-237.

*** Manual of the International Embryo Transfer Society (1998).